Polymerase Chain Reaction Detection Sensitivity of Gamma Irradiated *Bacillus* Spores

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Abstract

This study investigated the relationship between gamma irradiation levels and bacterial growth, polymerase chain reaction (PCR), and spore scanning electron microscopy of various preparations of *Bacillus thuringiensis* subsp. *kurstaki* spores. Four spore suspensions and one dry powder preparation were used, all of which were irradiated at eight different doses. PCR analysis was performed to determine if there were changes in sensitivity of the target sequence while enumeration and scanning electron microscopy (SEM) were used for determining viability and observing spore morphology, respectively.

Methods

Spore preparation

Five different preps were made as follows:

1) Dirty: spores were suspended in de-ionized sterile water.
2) Washed: spores were washed 6 times by centrifugation using water and then re-suspended in water.
3) Crude: spores were grown on agar plates and harvested.
4) Clean: spores were grown on agar plates, harvested, and washed 6 times with de-ionized sterile water.
5) Powder: spores were kept dry at 10^9 CFU/mL.

All liquid preparations were brought to 10^8 CFU/mL.

Gamma Irradiation

One mL (or 1g for powder) samples were subjected to 8 levels of gamma irradiation: (1) no radiation, (2) 1.5 x 10^6 rads, (3) 2.0 x 10^6 rads, (4) 2.5 x 10^6 rads, (5) 4.15 x 10^6 rads (ECBC minimum), (6) 5.43 x 10^6 rads (ECBC Standard), (7) 8.2 x 10^6 rads, and (8) 10.86 x 10^6 rads.

Real-time PCR analysis

PCR amplification reactions were carried out on the 7500 Fast Dx Real-time PCR instrument (Applied Biosystems).

Electron microscopy scanning of spores

Irradiated and non irradiated spores were observed with a scanning electron microscope.

Results

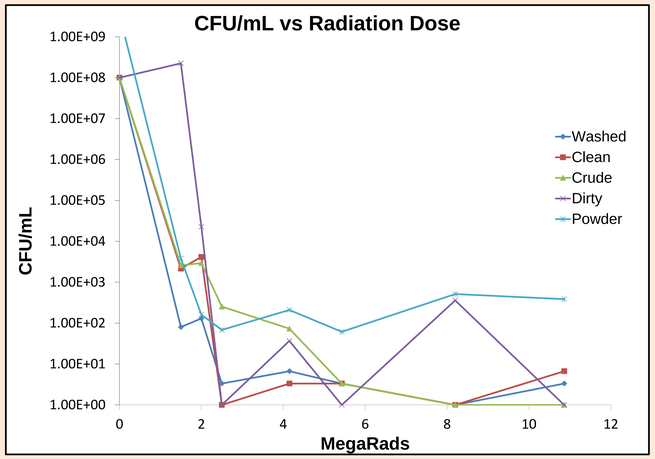

Figure 1. *Effect of gamma irradiation on the viability of various preparations of Bacillus thuringiensis spores. With the exception of some variation in the dirty prep, all inactivation curves have an initial swift decay rate followed by tail-off. At the range of 1.5 to 2.5 Mrad, a 6-log reduction of viable spores was achieved for all spore suspensions*

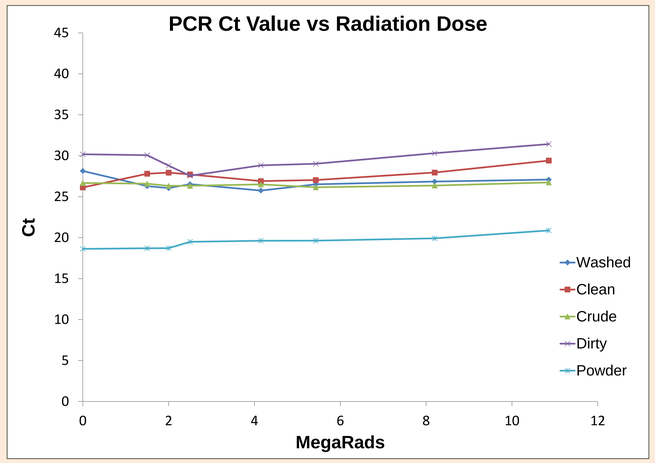

Figure 2. *Results depicting the effect of gamma radiation on PCR detection sensitivity.* All samples were detectable by PCR after gamma irradiation and Ct values of each prep remained constant regardless of irradiation dose.

Results

Spore Preparations	Gamma radiation [Mrad]							Mean (Ct)	SDV (Ct)
	[1.5]	[2]	[2.5]	[4.15]	[5.43]	[8.2]	[10.86]		
Washed	-1.85	-2.09	-1.61	-2.39	-1.62	-1.29	-1.05	-1.70	0.46
Dirty	-0.09	-1.37	-2.60	-1.34	-1.15	0.14	1.26	-0.74	1.26
Crude	-0.08	-0.35	-0.33	-0.16	-0.52	-0.31	0.08	-0.24	0.20
Powder	0.08	0.09	0.87	1.00	1.00	1.29	2.25	0.94	0.74
Cleaned	1.67	1.81	1.58	0.77	0.90	1.82	3.28	1.69	0.82

Table 1. *Effect of gamma irradiation on Bacillus thuringiensis PCR assay. These values indicate the differences of PCR threshold cycle (Ct) between treated [1-10.86 Mrad] and non treated spores. Gamma radiation decreased Ct value by as low as 2.60 cycles or increased the Ct by up to 3.28 cycles, but on average the Ct value was different by less than 2.*

Effect of gamma irradiation on PCR analysis of *B. thuringiensis* spores

The data were plotted as the average PCR cycle threshold (Ct) versus gamma irradiation dose (Mrads) for each spore preparation (Fig.2). When spores were prepared as crude, dirty, cleaned, or washed, the average Ct value for untreated spores was 27.77±1.46 versus 27.52±1.46 for irradiated spores. For powder spores, the average Ct value for untreated spores was 18.60± 0.00 versus 19.82±1.16 for irradiated spores. Based upon these data, gamma irradiation had no substantial effect on the sensitivity of PCR on the target sequences.

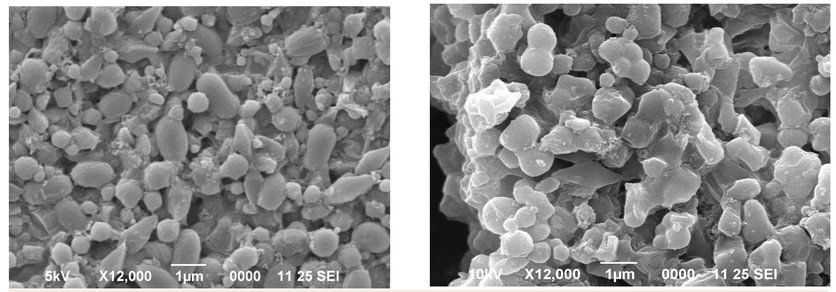

Figure 3. SEM image of B. thuringiensis at no radiation (left) and 10.86 Mrads radiation (right). Compared to non irradiated spores, most of the gamma-irradiated spores showed irregular, deformed shapes. The changes in spore morphology were revealed within 1.5-2.5 Mrad treatment which is the same dosage that caused a 6-log reduction in viability.

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